Construction of a Rapid-Mix Flow Cell for Sub-Millisecond Protein Folding Studies (2)

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Using a small volume mixer with the "continuous flow" method, the flow speed can be made very high, which accelerates the generation of turbulence in the sample flow and thus promotes efficient mixing. Dr. Denis Rousseau has developed such a mixer for resonance Raman and fluorescence spectroscopies, and we have modified the design for use with the synchrotron infrared beam. The mixer is designed such that two solutions are loaded into syringes and pumped at 2 mm/ms into a small mixing well (100 μm wide x 100 μm deep) etched into a stainless steel block. The mixing well is covered by a 50 μm-thick teflon sheet, which has a 200 μm-diameter hole aligned in the center of the mixing well. The solutions mix in the mixing well and flow through a dual-durometer seal into infrared observation cell, composed of two 12x25x2 mm long ZnSe windows. In one of them a 50 um deep, 200 um channel was engraved. By positioning the IR beam along the observation channel, different time points in the folding reaction were probed. The cell was tested by cytochrome–c folding and obtained time-constant for the reaction (~30 ms) agrees well with the literature data. The cell covers the time region from 1 to ~20 ms, and is currently redesigned for the use in both shorter (up to the dead time of the mixer, i.e. 100 us) and longer time scales (hundreds of ms).